Disruption of *ERBB2IP* Is not Associated with Dystrophic Epidermolysis Bullosa in Both Father and Son Carrying a Balanced 5;13 Translocation

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Mutations in the type VII collagen gene (*COL7A1*) cause autosomal recessive and autosomal dominant inherited dystrophic epidermolysis bullosa (DEB). We report a family with three individuals who present blistering, scarring, hypo- and hyperpigmentation, and nail dystrophy suggestive for DEB. Whereas father and son carry a 5;13 translocation, the daughter shows a normal karyotype. Segregation analysis revealed that all affected family members inherited the same *COL7A1* allele. Mutation analysis disclosed a heterozygous missense mutation, c.6227G>A (p.G2076D), in *COL7A1* in all affected individuals. Delineation of the translocation breakpoints showed that the *ERBB2IP* (erbb2 interacting protein or Erbin) gene is disrupted in 5q13.1 and *GPC6* in 13q32. *GPC6* encodes glypican 6 belonging to a family of cell surface heparan sulfate proteoglycans. The binding partners of Erbin, BP230 (BPAG1) and the integrin β4 subunit, both involved in hemidesmosome (HD) function, and the presence of Erbin in HD suggested that it plays a role in establishment and maintenance of cell-basement membrane adhesions. However, loss of function of one *ERBB2IP* copy or expression of a putative novel ERBB2IP fusion protein did not apparently modulate the DEB phenotype in both translocation patients. Nonetheless, one cannot yet exclude that *ERBB2IP* is a candidate for human blistering disorders such as epidermolysis bullosa.

Key words: disrupted gene/dystrophic epidermolysis bullosa/*ERBB2IP*/*GPC6*/translocation breakpoint
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Epidermolysis bullosa (EB) describes a group of clinically and genetically heterogenous disorders characterized by blistering of the skin and other epithelia. Patients with dystrophic EB (DEB) show mucocutaneous blistering, followed by extensive scarring and nail dystrophy (Bruckner-Tuderman, 1999). Autosomal dominant (DDEB) or autosomal recessive (RDEB) inheritance has been described for DEB; however, each form has its specific clinical presentation and severity. Both DDEB and RDEB are caused by mutations in the same gene, *COL7A1* in 3p21.1, encoding type VII collagen, the major component of anchoring fibrils (Bruckner-Tuderman, 1999). Although extensive clinical and molecular heterogeneities exist in DEB, no evidence for additional loci has yet been provided and all DEB variants seem to be allelic. Indeed, both the Pasini and Cockayne–Touraine variants of DEB have been shown to be caused by missense mutations in *COL7A1* (Kon *et al*, 1997). In general, the type and combination of *COL7A1* mutations may predict the clinical severity and progression of the DEB phenotype (Uitto and Richard, 2004).

Here we describe a family with three members affected by DDEB and two, father and son, carrying a 5;13 translocation that disrupts the *ERBB2IP* gene in 5q13. *ERBB2IP* encodes the basolateral protein Erbin that binds to the 230-kDa bullous pemphigoid antigen (BP230/BPAG1) and the integrin β4 subunit, suggesting that Erbin may be involved in regulating the assembly of hemidesmosomes (HD).

Abbreviations: DEB, dystrophic epidermolysis bullosa; FISH, fluorescent *in situ* hybridization; HD, hemidesmosome

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Results and Discussion

In a five-generation extended family (Fig 1*A*), four individuals (II-1, III-3, IV-2, and IV-5) in three generations were affected by skin blistering. Based on the clinical phenotype and the family history, an autosomal dominant DEB-type Cockayne–Touraine was assumed to segregate in all affected family members.

To identify the causative mutation in the affected persons, we performed haplotype analysis using polymorphic DNA markers closely linked to known EB loci. As no immunohistological analyses of the patients' skin were available, we could not formally exclude the possibility that the affected persons presented an EB subtype that differs from DEB, such as e.g., EB simplex. Segregation analysis excluded an involvement of the genes *KRT5* and *KRT14* in the EB phenotype of the patients (data not shown). In contrast, haplotype analysis of the *COL7A1*-harboring allele showed that the same disease allele is present in all affect-

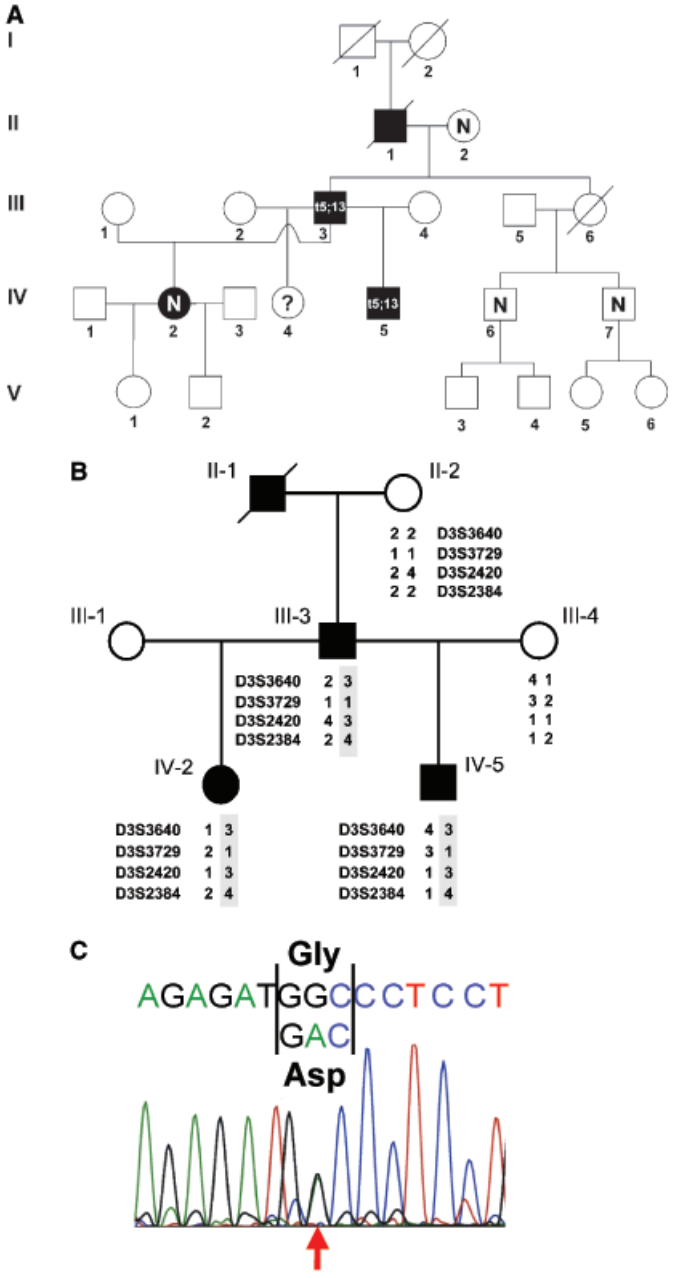

Figure 1
Identification of a pathogenic missense mutation in *COL7A1* in three affected family members. (*A*) Pedigree of the five-generation Bulgarian family. Individuals carrying the 5;13 translocation (t5;13) as well as those with a normal karyotype (N) are indicated. The karyotype of individual IV-4 is not known (?). (*B*) Partial pedigree of the family and segregation analysis of *COL7A1* alleles. The *COL7A1* gene is located between markers D3S2420 and D3S2384. All affected persons share one allele (gray box) that was not inherited from the healthy grandmother. (*C*) A heterozygous p.G2076D missense mutation in *COL7A1* in the family members affected by DEB. Electrophortogram of a part of *COL7A1* exon 75. Direct sequencing of the exon 75 amplicon revealed a heterozygous base change, c.6227G>A (indicated by a red arrow), causing an amino acid change from glycine (Gly) to aspartic acid (Asp).

ed members (Fig 1*B*). Subsequently, we identified a heterozygous missense mutation in exon 75 of *COL7A1*, c.6227G>A (p.G2076D), in the three affected individuals (III-3, IV-2, and IV-5) (Fig 1*C*). This mutation has been previously reported to be associated with the Pasini type of autosomal dominant DEB (Kon *et al*, 1997), indicating that the *COL7A1* mutation is causative for the disease phenotype in this family. Comparison of expected and observed mutations in *COL7A1* in DEB patients revealed that substitution of any glycine by different amino acids will cause disease, supporting the notion that this missense mutation is indeed pathogenic (Persikov *et al*, 2004).

Remarkably, conventional cytogenetic analysis revealed that both the affected father (III-3) and his son (IV-5) carry an apparently balanced translocation between chromosomes 5 and 13, 46,XX,t(5;13)(q13;q32) (Fig 2*A*). The daughter (IV-2), however, showed a normal karyotype, suggesting that the translocation is not primarily associated with the disease phenotype in the family. Individuals II-2, IV-6, and IV-7 show a normal karyotype whereas individual IV-4 has not been investigated. Taken together, these findings indicate that the c.6227G>A mutation in *COL7A1* is causative for the skin disorder, although we cannot yet exclude that disruption of a gene(s) in the breakpoint region(s) may have a modulating effect on the phenotype. Therefore, we delineated the breakpoint regions of the 5;13 translocation by fluorescence *in situ* hybridization (FISH), and breakpoint spanning BAC clones were identified for both breakpoint regions. For 5q13, BAC CTD-2033C11 showed split signals on both derivative chromosomes 5 and 13 (Fig 2*B*), whereas RP11-158B14 overlaps the 13q32 breakpoint (Fig 2*C*). By database searches, we found that almost the entire *ERBB2IP* (*erbb2 interacting protein*) gene is located on the insert of CTD-2033C11, suggesting that the breakpoint directly disrupts *ERBB2IP* (Fig 2*D*). The breakpoint spanning clone RP11-158B14 harbors exons 5 and 6 of the *GPC6* (*glypican 6*) gene that consists of nine exons in total, indicating that this gene is interrupted by the translocation breakpoint in 13q32 (Fig 2*D*). Indeed, we confirmed disruption of both genes by generating various *ERBB2IP–GPC6* fusion transcripts from RNA isolated from the two translocation patients (data not shown) that were not amplified from RNA of control persons. In the most abundant fusion transcript, exons 1–7 of *ERBB2IP* were spliced to *GPC6* exons 6–8; however, no *ERBB2IP–GPC6* fusion protein is generated from this transcript as the open reading frame (ORF) of *ERBB2IP* exon 7 ends with the second base of a triplet whereas the ORF of *GPC6* exon 6 begins with the first nucleotide of a triplet. Thus, a putative novel ERBB2IP protein could be produced consisting of the N-terminal 178 amino acids of Erbin and 51 unrelated amino acids encoded by a part of *GPC6* exon 6 (Fig 2*E*). Together, the data suggest that the translocation breakpoint in 5q13 is located in intron 7 of *ERBB2IP* and in *GPC6* intron 5 in 13q32.

GPC6 encodes a new member of the glypican family of cell surface heparan sulfate proteoglycans (Paine-Saunders *et al*, 1999; Veugelers *et al*, 1999). Glypicans play a role in developmental morphogenesis, e.g., mutations in the X-linked *GPC3* gene cause the Simpson–Golabi–Behmel syndrome (SGBS), an overgrowth syndrome (Pilia *et al*, 1996). Males typically affected by SGBS show tall stature, coarse face, supernumerary nipples, congenital heart defect, and generalized muscular hypotonia (Neri *et al*, 1998). The two male patients affected by DEB do not show any symptoms typical for SGBS.

ERBB2IP codes for a protein called Erbin, originally identified as a binding partner of the ERBB2/HER2 receptor (Borg *et al*, 2000). Erbin belongs to the LAP (leucine-rich repeats and PDZ domains) protein family, critically involved in establishing cell polarity (Santoni *et al*, 2002). Various binding partners of Erbin have been identified, as e.g., p0071 (plakophilin-4), BP230 (BPAG1), and the β4 subunit of integrins (Favre *et al*, 2001; Jaulin-Bastard *et al*, 2002). p0071 is a protein linked to the cytoskeleton that colocalized with Erbin in desmosomes of human colon cells (Jaulin-Bastard *et al*, 2002). BP230 (230-kDa bullous pemphigoid antigen/BPAG1) is a component of HD. These are specialized transmembrane cell–matrix junctions that mediate the contact between basal keratinocytes and the basement membrane. Additional HD components are the α6β4 integrins and collagen XVII, also called the 180-kDa bullous pemphigoid antigen or bullous pemphigoid antigen 2 (BP180/BPAG2/COL17A1), both transmembrane molecules associated with HD that link the intermediate filament cytoskeleton to the extracellular matrix of the basement membrane (Jonkman, 1999). HD are important for the integrity of the dermal–epidermal junction, and any disturbance resulting in either the absence of one of the HD components or causing altered protein–protein interactions can lead to autosomal recessively inherited hemidesmosomal EB variants. For example, mutations in *ITGA6* and *ITGB4*, encoding α6β4 integrin, have been identified in patients with junctional EB with pyloric atresia (Vidal *et al*, 1995; Pulkkinen *et al*, 1997). Moreover, mutations in the BPAG2-encoding gene *COL17A1* were found in patients with generalized atrophic benign EB (McGrath *et al*, 1995). Consequently, the presence of Erbin in desmosomes and HD suggested that it may play a role in the establishment and maintenance of cell–cell and cell–basement membrane adhesions. This hypothesis is supported by the finding that mice deficient for the scaffold protein GRIP1, which contains seven PDZ domains, show extensive skin blistering reminiscent of DEB (Bladt *et al*, 2002). Although no disease-causing mutations have yet been found in *BPAG1*, *ERBB2IP*, or *GRIP1*, it is tempting to speculate that these genes are obvious candidates for human blistering skin disorders such as EB. Consequently, we assumed that disruption of *ERBB2IP* in both father (III-3) and son (IV-5) with DEB may modulate their phenotype. Therefore, we compared the phenotype of in-

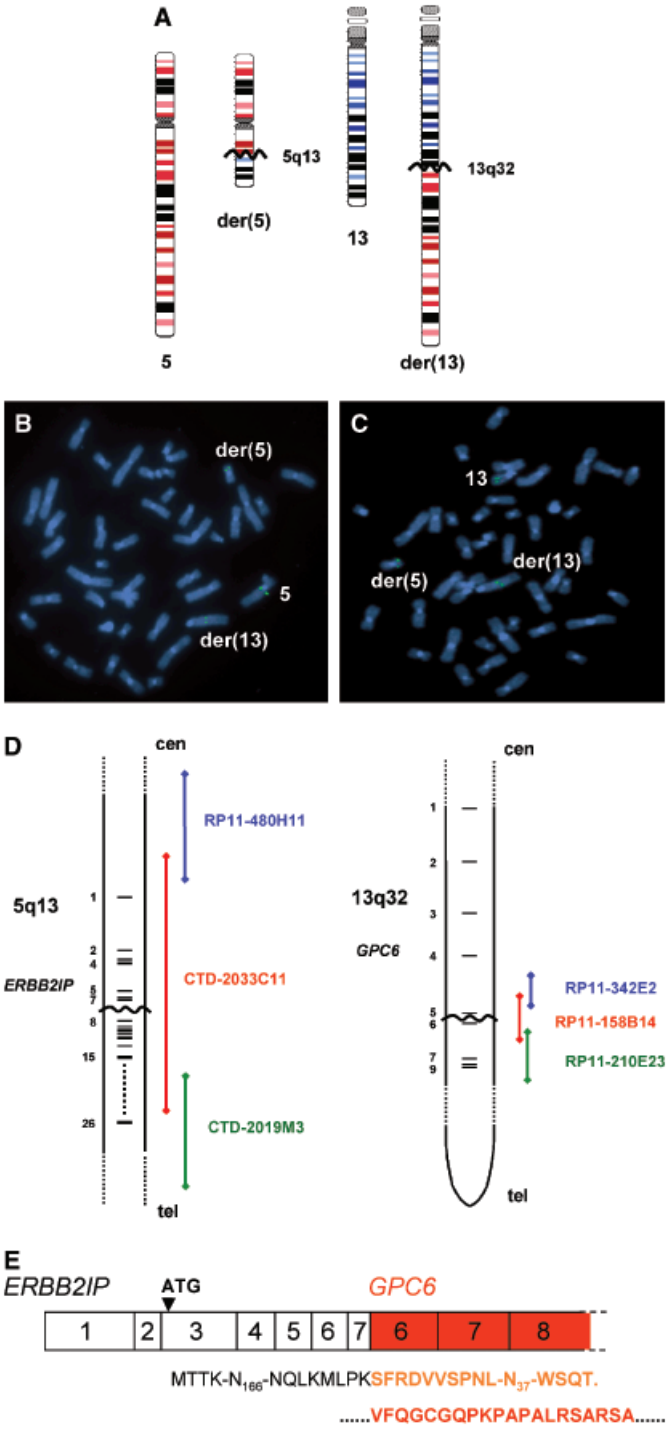

Figure 2
The translocation disrupts *ERBB2IP* in 5q13 and *GPC6* in 13q32. (*A*) Schematic representation of the 5;13 translocation. The ideograms of the normal and derivative (der) chromosomes 5 and 13 are shown. The breakpoints at 5q13 and 13q32 are indicated by wavy lines. (*B* and *C*) Identification of overlapping BAC clones for the breakpoint regions in 5q13 and 13q32 by fluorescence *in situ* hybridization (FISH). (*B*) FISH with BAC clone CTD-2033C11 hybridized to lymphocyte metaphase spreads of patient IV-5. The probe is labeled by fluorescein isothiocyanate (green). BAC CTD-2033C11 shows signals on wild-type chromosome 5, der(5), and der(13). (*C*) FISH with BAC clone RP11-158B14 on lymphocyte metaphase spreads of individual IV-5 that produces a signal (green) on wild-type chromosome 13 and on both derivative chromosomes [der(5) and der(13)]. Chromosomes are counterstained with diamidinophenyl indole. (*D*) Schematic representation of the breakpoint regions in 5q13 (*left*) and 13q32 (*right*). Chromosomal parts of 5q13 and 13q32, respectively, are shown schematically. BAC clones used for FISH are indicated by colored bars: BACs hybridizing proximal to the breakpoint are indicated in blue, those hybridizing distal are shown in green, and breakpoint spanning clones are shown in red. Names of BACs are given. Exons of *ERBB2IP* and *GPC6* are represented by black bars and are partially numbered on the left. Breakpoints are indicated by wavy lines. (*E*) Schematic representation of an *ERBB2IP–GPC6* fusion transcript identified in the translocation patients. Exons are indicated by boxes and numbered; white boxes represent various *ERBB2IP* exons whereas red boxes represent those of the *GPC6* gene. The ATG start codon of *ERBB2IP* in exon 3 is indicated. ERBB2IP amino acids (single letter code) up to the end of exon 7 are shown in black: amino acid residues encoded by *GPC6* from exon 6 onward are shown in red. The residues of the novel 51 amino acid peptide resulting from the translocation (encoded by *GPC6* exon 6 but in a different open reading frame) are shown in orange.

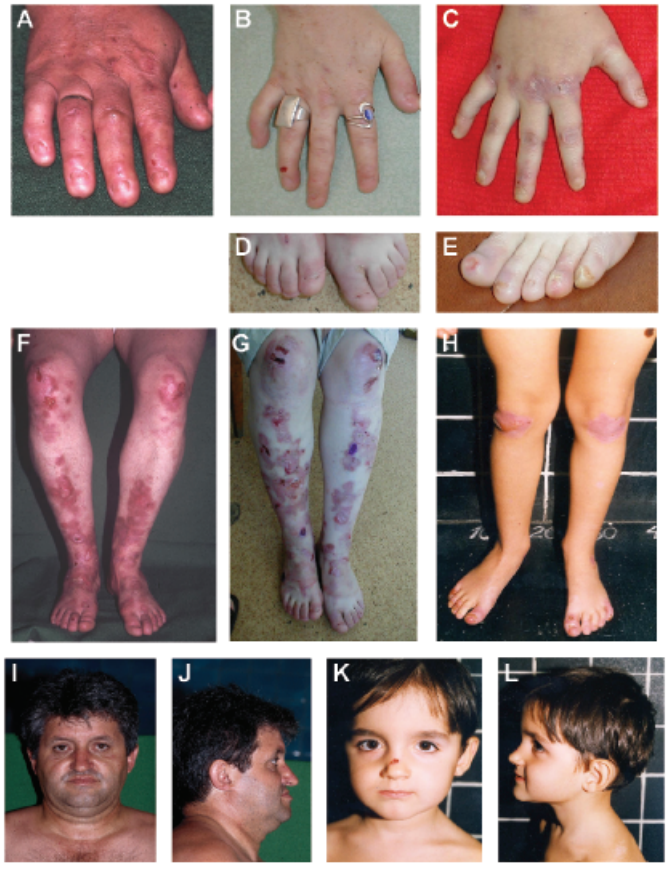

Figure 3
Representative photographs of the family members affected by dystrophic epidermolysis bullosa. Hand of individual III-3 (*A*), that of the daughter of III-3 (IV-2) (*B*), and one hand of the 9-y-old son of patient III-3 (IV-5) (*C*). Toenails of patients IV-2 (*D*) and IV-5 (*E*). Pretibial region of individual III-3 (*F*), his daughter (*G*), and son (*H*). Facial appearance of patient III-3 (*I* and *J*) and his son (IV-5) (*K* and *L*). Note hypoplasia of the maxilla in both individuals. For detailed description of the photos, see text.

dividuals III-3 and IV-5 with that of IV-2 showing a normal karyotype.

Patient III-3 (58 y old; height: 168 cm), the father of patients IV-2 and IV-5, presented skin lesions affecting predominantly the hands and knees as well as the pretibial regions (Fig 3*A* and *F*). Blisters healed with scarring, skin atrophy, and hypo- and hyperpigmentation (Fig 3*F*). His toe nails showed dystrophy and some of them were completely lost whereas all fingernails were lost (Fig 3*A*). The daughter from the first marriage of individual III-3 (IV-2) is 31 y old. She has been initially seen by one of us (M. S.) at the age of 17. Soon after birth she developed painful bullous skin lesions, mainly on distal parts of arms and legs as well as elbows and knees (Fig 3*B* and *G*). The lesions were caused even by mild injuries and healed very slowly with scars; her nails were dystrophic or lost (Fig 3*B* and *D*). There was no other organ involvement. The 9-y-old son of patient III-3 (IV-5) was referred to the Clinical Genetics Unit because of skin blistering starting at day 2 postnatal. He was born to a healthy mother of caucasian origin (III-4), after Cesarean section because of fetal cardiac decelerations at 39 wk of gestation. Birth weight and length were normal. Bullous skin lesions occurred spontaneously; they had a serous or hemorrhagic content. There was evidence, however, for blistering exacerbation after mechanical trauma. All blisters were localized over the dorsal part of the hands and feet and on the lower legs (Fig 3*C* and *H*), whereas other regions as well as the trunk, limbs, eyes, and the oral mucosa were unaffected. Long-term follow-up of patient IV-5 showed a normal mental and physical development (height: 120 cm at the age of 7.5 y). He underwent treatment either systemic with vitamin E or/and topical with emollients and corticosteroids without any significant clinical improvement. Until the age of 4.5 y, there was a slow expansion and progression of the lesions; nails became dystrophic (Fig 3*E*) and skin scars and areas of hypo- and hyperpigmentation developed on the distal parts of the limbs (Fig 3*H*) similar to those observed in the father. Anamnestically, the deceased father of individual III-3 (II-1) had similar skin lesions. Patient III-3 as well as his son (IV-5) neither have oral problems nor anemia. Taken together, the clinical phenotypes of the three affected persons are very similar, showing no significant differences that might be associated with different karyotypes. But both father (III-3) and son (IV-5) carrying the 5;13 translocation show a mild facial dysmorphism, e.g., hypoplasia of the maxilla (Fig 3*I–L*).

In light of the skin disease segregating in the family described here, interruption of *ERBB2IP*, encoding a protein possibly implicated in HD function, is challenging. Nonetheless, the *COL7A1* missense mutation p.G2076D is most likely associated with DEB in the affected members of the family described here. Moreover, loss of function of one *ERBB2IP* copy or, alternatively, expression of a putative novel ERBB2IP fusion protein that might act in a dominant-negative manner does not seem to modulate the EB phenotype in father and son. One cannot, however, exclude that disruption of *ERBB2IP* as well as missense, nonsense, or splice mutations in this gene are implicated in EB.

Materials and Methods

Patients A five-generation Bulgarian family (Fig 1*A*) with three individuals (III-3, IV-2, and IV-5) in two generations who were affected by skin blistering as well as four unaffected individuals (II-2, III-4, IV-6, and IV-7) were included in this study. Peripheral blood samples were taken from patients for DNA isolation and chromosome preparations in adherence to the Declaration of Helsinki Guidelines, following their informed consent and the relevant ethics committee approval for this study.

Microsatellite typing PCR amplification for the analysis of microsatellite markers was performed on genomic DNA from individuals II-2, III-3, III-4, IV-2, and IV-5. Genomic DNA was isolated by standard procedures. Haplotype analysis of the *KRT14* allele was carried out with D17S846, D17S1563, and D17S967, with D12S297, D12S368, D12S390, and D12S766 for the *KRT5* allele, and with D3S3640, D3S3729, D3S2420, and D3S2384 for the *COL7A1* allele. Primer sequences and PCR conditions are available on request. Amplification products were electrophoresed on 8% polyacrylamide gels at 400 V for $\sim$ 2 h. Subsequently, bands were detected by silver staining.

Mutation analysis of *COL7A1* Mutation detection was performed by direct sequencing of the coding region of the *COL7A1* gene. For amplification of all 118 *COL7A1* exons and exon/intron boundaries, 73 pairs of primers were designed according to Christiano *et al* (1997) with modifications. For exons 73–75 the following PCR

primers were used: 5′-CCACCAGCATTCTCTCTTCCA-3′ and 5′-TGGCTTCCTGGTCACTAGTCA-3′ generating a product of 556 bp; sequencing primers were 5′-AGCCTGGAAAGCCTGGTATT-3′, and 5′-ACAGGACTAAGGCAGGGATG-3′. PCR products were directly sequenced with the internal primers listed above using the BigDye Terminator Kit 1.1 (Applied Biosystems, Darmstadt, Germany). Samples were analyzed on an ABI 3100 automated sequencer (Applied Biosystems).

Conventional cytogenetic and FISH studies Chromosome preparations were made from phytohemagglutinin-stimulated peripheral blood lymphocytes. Conventional cytogenetic analysis of G-banded chromosomes was carried out by high-resolution banding (500-band level). FISH was performed as previously described (Kutsche *et al*, 2000). Briefly, BAC DNA were labeled with biotin-16-dUTP by nick translation (Roche, Mannheim, Germany). Fluorescein isothiocyanate-labeled streptavidin (1:200) was used for detection. Chromosomes were counterstained using diamidinophenyl indole (Roche) and mounted in antifading solution (Vector Labs, Burlingame, California). Slides were analyzed with a Leica Axioscope fluorescence microscope. Images were merged using a cooled CCD camera (Pieper, Schwerte, Germany) and Cyto Vision (Applied Imaging, San Jose, California) software.

BAC clones BAC clones RP11-342E2, RP11-145B14, RP11-210E23, and RP11-480H11 were provided by the Resource Center of the German Human Genome Project at the Max-Planck-Institute for Molecular Genetics, Berlin, Germany whereas BAC clones CTD-2033C11 and CTD-2019M3 were obtained from Invitrogen (Karlsruhe, Germany). BAC DNA were prepared using a Midiprep kit (Qiagen, Hilden, Germany).

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